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Bioactive Coating Systems for Protection Against Bio-threats: Antimicrobial Coatings for Medical Shelters

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14. ABSTRACT

Over the course of this program, the Center for Nanoscale Science and Engineering (CNSE) at North Dakota State University (NDSU)—in partnership with Triton Systems, Inc.—augmented its core materials science research capabilities to foster the development of next generation, antimicrobial coating technologies aimed at protecting US military personnel from exposure to hazardous biological agents in the battlefield. A key element to the success of this project was the development, early on, of a high-throughput biological screening workflow to enable combinatorial exploration of novel antimicrobial coating/treatment concepts. A number of different strategies

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| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | Bret Chisholm |
| UU | UU | UU | UU | | 19b. TELEPHONE NUMBER 701-231-5328 |

## Report Title

Bioactive Coating Systems for Protection Against Bio-threats:
Antimicrobial Coatings for Medical Shelters

## ABSTRACT

Over the course of this program, the Center for Nanoscale Science and Engineering (CNSE) at North Dakota State University (NDSU)—in partnership with Triton Systems, Inc.—augmented its core materials science research capabilities to foster the development of next generation, antimicrobial coating technologies aimed at protecting US military personnel from exposure to hazardous biological agents in the battlefield. A key element to the success of this project was the development, early on, of a high-throughput biological screening workflow to enable combinatorial exploration of novel antimicrobial coating/treatment concepts. A number of different strategies based on reactive, functional oligomers containing quaternary ammonium salts (QAS) were investigated for their ability to impart antimicrobial properties to both fabrics (i.e., nylon and polyester) and other rigid materials (i.e., glass and metals) of relevance to the US military. One approach in particular, based on QAS-functional acrylates, was shown to be highly effective at generating broad-spectrum, antimicrobial treatments for polyester fabric using Triton Systems novel atmospheric pressure plasma deposition process (Invexus™). It is envisioned that these new antimicrobial technologies developed at NDSU will be harnessed by Triton Systems to produce efficacious and operationally functional products for the US military via their industrial scale, textile treatment line (RC1000™).

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## Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

| Received | | Paper |
|---|---|---|
| 08/23/2012 | 1.00 | M.R. Bayati, P.E. Petrochenko, S. Stafslien, J. Daniels, N. Cilz, D.J. Comstock, J.W. Elam, R.J. Narayan, S.A. Skoog. Antibacterial activity of zinc oxide-coated nanoporous alumina, Materials Science and Engineering: B, (07 2012): 0. doi: 10.1016/j.mseb.2012.04.024 |
| 08/27/2012 | 2.00 | Philip R Miller, Ritika Singh, Akash Shah, Shane Stafslien, Justin Daniels, Roger J Narayan, Ryan D Boehm. Indirect rapid prototyping of antibacterial acid anhydride copolymer microneedles, Biofabrication, (03 2012): 0. doi: 10.1088/1758-5082/4/1/011002 |
| **TOTAL:** | **2** | |

**Number of Papers published in peer-reviewed journals:**

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### (b) Papers published in non-peer-reviewed journals (N/A for none)

| Received | | Paper |
|---|---|---|
| **TOTAL:** | | |

**Number of Papers published in non peer-reviewed journals:**

## (c) Presentations

**Number of Presentations:** 0.00

### Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

| Received | Paper |
|---|---|

**TOTAL:**

**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

### Peer-Reviewed Conference Proceeding publications (other than abstracts):

| Received | Paper |
|---|---|

**TOTAL:**

**Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## (d) Manuscripts

| Received | Paper | |
|---|---|---|
| 12/23/2013 | 3.00 | Ryan Boehm, Philip R. Miller, Justin Daniels, Shane Stafslien, Roger J. Narayan. Inkjet Printing for Pharmaceutical Applications, Materials Today (09 2013) |

**TOTAL:** **1**

**Number of Manuscripts:**

## Books

| Received | Paper |
|---|---|

**TOTAL:**

## Patents Submitted

## Patents Awarded

## Awards

None

## Graduate Students

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Post Doctorates

| NAME | PERCENT_SUPPORTED |
|---|---|
| Satyabrata Samanta | 0.02 |
| **FTE Equivalent:** | **0.02** |
| **Total Number:** | **1** |

## Names of Faculty Supported

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Under Graduate students supported

| NAME | PERCENT_SUPPORTED | Discipline |
|---|---|---|
| Ashley A. Breiland | 0.51 | Geology |
| Anurad G.J. Jayasooriya M. | 0.02 | Microbiology |
| Brandon N. Kuntz | 1.00 | None Declared |
| Mary E. Luther | 0.83 | N/A |
| Andrew J. Muehlberg | 0.70 | Microbiology |
| Jaboc A. Steiner | 0.84 | Biochemistry/Molecular Biology |
| **FTE Equivalent:** | **3.90** | |
| **Total Number:** | **6** | |

## Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

| | |
|---|---|
| The number of undergraduates funded by this agreement who graduated during this period: ..... | 2.00 |
| The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... | 2.00 |
| The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... | 1.00 |
| Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... | 1.00 |
| Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... | 0.00 |
| The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... | 0.00 |
| The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... | 0.00 |

## Names of Personnel receiving masters degrees

| NAME |
|---|
| **Total Number:** |

## Names of personnel receiving PHDs

| NAME |
|---|
| **Total Number:** |

## Names of other research staff

| NAME | PERCENT_SUPPORTED |
|---|---|
| James Bahr | 0.22 |
| Justin Daniels | 0.52 |
| Shane Stafslien | 0.60 |
| Michael Weisz | 0.28 |
| Bret Chisholm | 0.08 |
| **FTE Equivalent:** | **1.70** |
| **Total Number:** | **5** |

## Sub Contractors (DD882)

1 a. Triton Systems, Incorporated

1 b. 200 Turnpike Road

Chelmsford MA 01824

**Sub Contractor Numbers (c):**

**Patent Clause Number (d-1):**

**Patent Date (d-2):**

**Work Description (e):**

**Sub Contract Award Date (f-1):**

**Sub Contract Est Completion Date(f-2):**

## Inventions (DD882)

## Scientific Progress

See attached pdf document entitled "W911NF-10-1-0519 Final Report"

## Technology Transfer

# Bioactive Coating Systems for Protection Against Bio-Threats: Antimicrobial Coatings for Medical Shelters

## Final Report


Grant: **W911NF-10-1-0519**

Program Period of Performance**:** **01 October 2010 through 30 September 2013**

Prepared for: U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

ARO Grant Officer: Dr. Jennifer J. Becker
Chemical Sciences Division



Prepared by: **Bret Chisholm, Shane Stafslien, James Bahr and Satyabrata Samanta**
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Chelmsford, MA 01824-4000



Date: **24 December 2013**

## Abstract

Over the course of this program, the Center for Nanoscale Science and Engineering (CNSE) at North Dakota State University (NDSU)—in partnership with Triton Systems, Inc.—augmented its core materials science research capabilities to foster the development of next generation, antimicrobial coating technologies aimed at protecting US military personnel from exposure to hazardous biological agents in the battlefield. A key element to the success of this project was the development, early on, of a high-throughput biological screening workflow to enable combinatorial exploration of novel antimicrobial coating/treatment concepts. A number of different strategies based on reactive, functional oligomers containing quaternary ammonium salts (QAS) were investigated for their ability to impart antimicrobial properties to both fabrics (i.e., nylon and polyester) and other rigid materials (i.e., glass and metals) of relevance to the US military. One approach in particular, based on QAS-functional acrylates, was shown to be highly effective at generating broad-spectrum, antimicrobial treatments for polyester fabric using Triton Systems novel atmospheric pressure plasma deposition process (Invexus™). It is envisioned that these new antimicrobial technologies developed at NDSU will be harnessed by Triton Systems to produce efficacious and operationally functional products for the US military via their industrial scale, textile treatment line (RC1000™).

# Table of Contents

## 1.0 Statement of the Problem Being Studied

This project seeks to establish a systematic approach for developing new antimicrobial coatings with relevance to the individual and collective protection equipment. Such coatings may help to prevent infections to injured military personnel caused by difficult-to-treat bacteria such as *Acinetobacter baumannii* and Methicillin-Resistant *Staphylococcus aureus* (MRSA). To reduce medical-care-acquired infections, clean environments are critical. Operating rooms in the Army's Deployable Medical System (DEPMEDS) are routinely scrubbed and cleaned. However, surges of casualties during a high intensity conflict would not generally allow a thorough cleaning of the surgical area between operations. Antimicrobial coating development and efficacy is a complex process and depends on the substrate of interest, the method by which active ingredients are incorporated, bio-agent composition, conditions under which treated parts are used and weathered, and testing protocols. This project is estabilishing a combinatorial workflow for coating development and testing, which will allow us to better understand coating-performance relationships and enable a shorter product development cycle.

Another important element of this project, besides the combinatorial approach, is the Atmospheric Pressure Plasma Liquid Deposition (APPLD) technology by which active ingredients are deposited on various surfaces. Unlike most other methods, APPLD will seamlessly incorporate active ingredients on the very top surface of almost any substrate. This novel technology has the potential to improve antimicrobial efficacy due to higher surface concentration and better bonding of active ingredients to the surface. Antimicrobial coatings deposited by APPLD have been shown to reduce bacterial colonization on a broad range of materials and equipment including sensitive electronics which cannot be coated using conventional coating processes. Furthermore, APPLD has been shown to provide antimicrobial protection to optical systems, computer screens, and equipment monitors which require transparency. Operating at ambient temperature and atmospheric pressure, the APPLD process enables deposition of durable antimicrobial coatings without degradation of substrate properties.

## 2.0 Summary of Technical Progress - NDSU

The following text provides a detailed overview of technical progress made in the final year of this project (October 1st, 2012 to September 30, 2013) and a concise summary of the accomplishments and conclusions made since the inception of the program in the fall of 2010.

## 2.1 Establish the Combinatorial/High-Throughput Workflow

### 2.1.1 Multi-species Bacterial Aerosol Deposition and Analysis

The aerosol-based antimicrobial screening workflow was augmented during the final year of this project to facilitate the simultaneous deposition and analysis of two bacterial species on a single array of samples. As shown in Figure 1, the modified screening methodology relies on the use of antimicrobial treated discs with a smaller footprint than utilized previously (i.e., 10 mm vs. 15 mm). This reduced sample size allows the same number of unique treatments (six total; rows) and assay replicates (three total; columns (R1, R2 and R3)) to be evaluated for each array plate as the original testing method—but now for two bacterial isolates instead of a single species. In the example provided in Figure 1, a co-culture of the Gram-negative bacterium, *Escherichia coli*, and the Gram-positive bacterium, *Staphylococcus aureus*, were aerosolized and deposited onto an array of non-treated aluminum discs. The left half of the array plate (columns 1 – 4) was covered with a slab of agar specifically formulated to select for the growth of *E. coli*, only, by using an inhibitory concentration of the respiratory indicator dye, triphenyl tetrazolium chloride (TTC), for *S. aureus.* Conversely, the right half of the array plate (columns 5 – 8) was overlaid with phenylethyl alcohol agar to prevent the growth of *E. coli*, but allow for the growth of *S. aureus.*

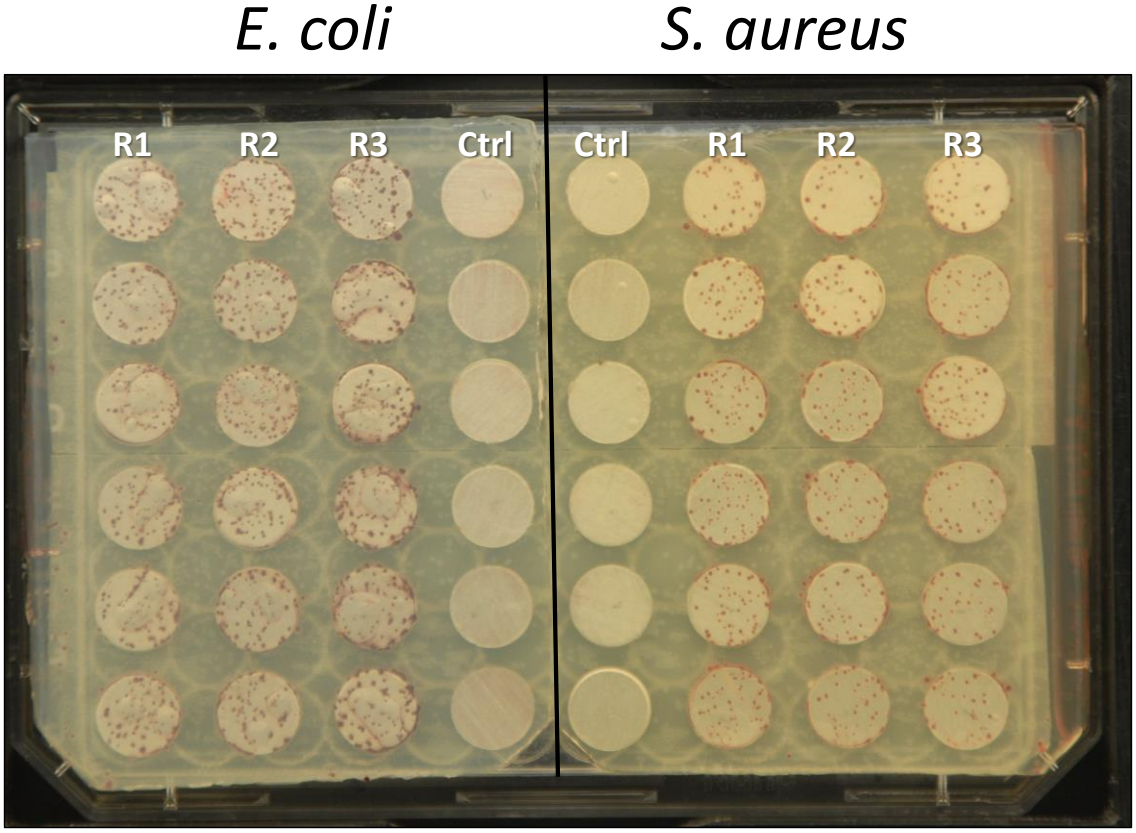


**Figure 1.** Image of *E. coli* and *S. aureus* growth (red color; 18 hours @ 37ºC) after co-culture deposition onto un-treated aluminum discs applied to a single array plate. R1 = replicate 1, R2 = replicate 2, R3 = replicate 3 and Ctrl = assay control (no bacteria).

Similar to the screening method based on antimicrobial-treated discs, the fabric-based assay was also modified to accommodate the simultaneous evaluation of two bacterial species for each array of samples. A rubber gasket was placed across the middle of fabric strips to prevent bacterial deposition and served as assay control region (Figure 2). Top half of the plate received the appropriate slab of agar (described above) to select for the growth of *E. coli* while the bottom half of the plate received the agar slab to select for the growth of *S. aureus*. As with the disc-based testing method, the same number of unique treatments and assay replicates can be evaluated as with the original screening method for one species.

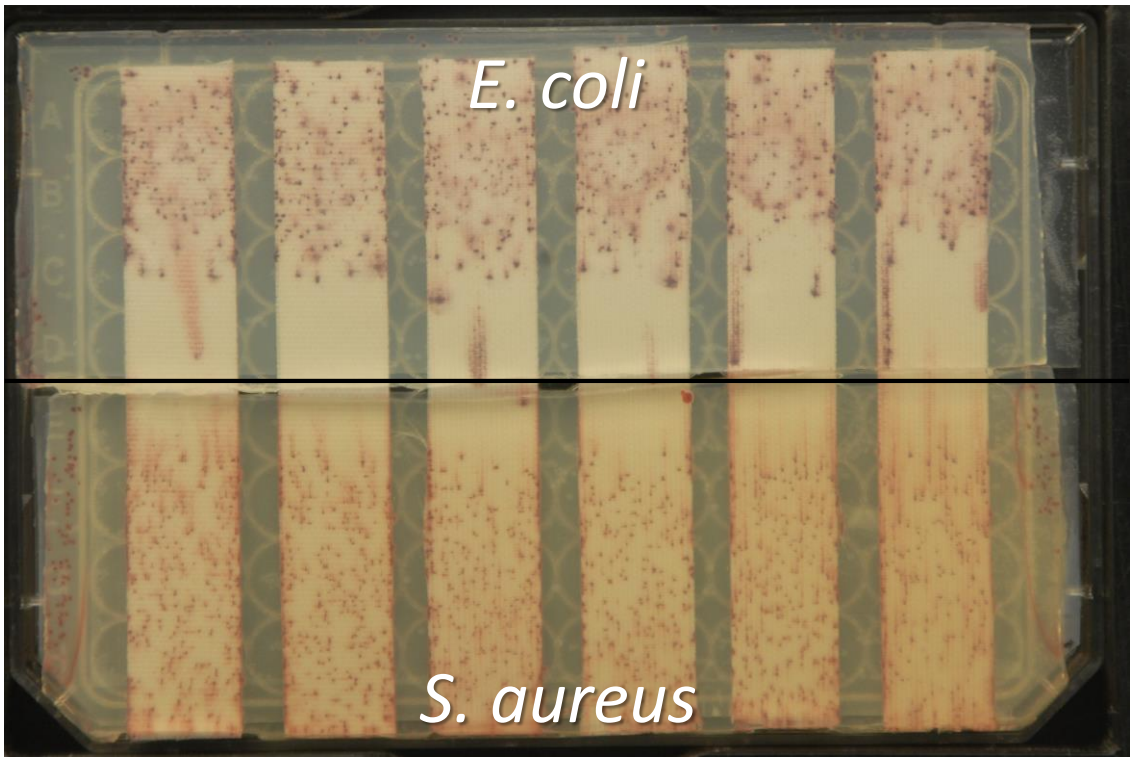


**Figure 2.** Image of *E. coli* and *S. aureus* growth (red color; 18 hours @ 37ºC) after co-culture deposition onto strips of un-treated fabric applied to a single array plate. Control region (i.e., no bacterial deposition) was created in the middle of the plate by placing a rubber gasket across the fabric strips during the aerosol deposition procedure.

### 2.1.2 Triton PlasmaJet Coating Platform

After the completion of the APPLD PlasmaJet coating system by Triton, NDSU researchers were trained on the use of the tool at Triton. At this time various materials were deposited onto both glass and fabric substrates in order to optimize the deposition parameters. The goal of this activity was to achieve a uniform coating across the substrate. It was discovered that solutions containing polymers tended to plug the atomization nozzle after a few minutes of deposition. From this point on, only low molecular weight monomers were used for deposition. Other difficulties encountered had to do with the buildup of atomized liquid on the inside of the plasma nozzle that led to large droplets of monomer solution dripping off of the nozzle onto the substrate.

At the end of the training, the APPLD was transferred to NDSU and installed in the Combinatorial Materials Research Lab. We then modified the deck of the tool so that it would receive our standard substrates with the addition of linear brushes on either side to wipe off any drips before they fell onto the substrate. We also added a second syringe pump and valving system to allow the nozzle to be flushed with solvent as soon as the plasma deposition process

has completed. Prior to this addition, several minutes would pass before it was safe to open the enclosure after a deposition in order to clean and flush the nozzle. This sometimes resulted in the plugging of the nozzle as the monomer solution dried on the tip. With the APPLD PlasmaJet installed at NDSU, all of the components of the antimicrobial fabric workflow are now in place.

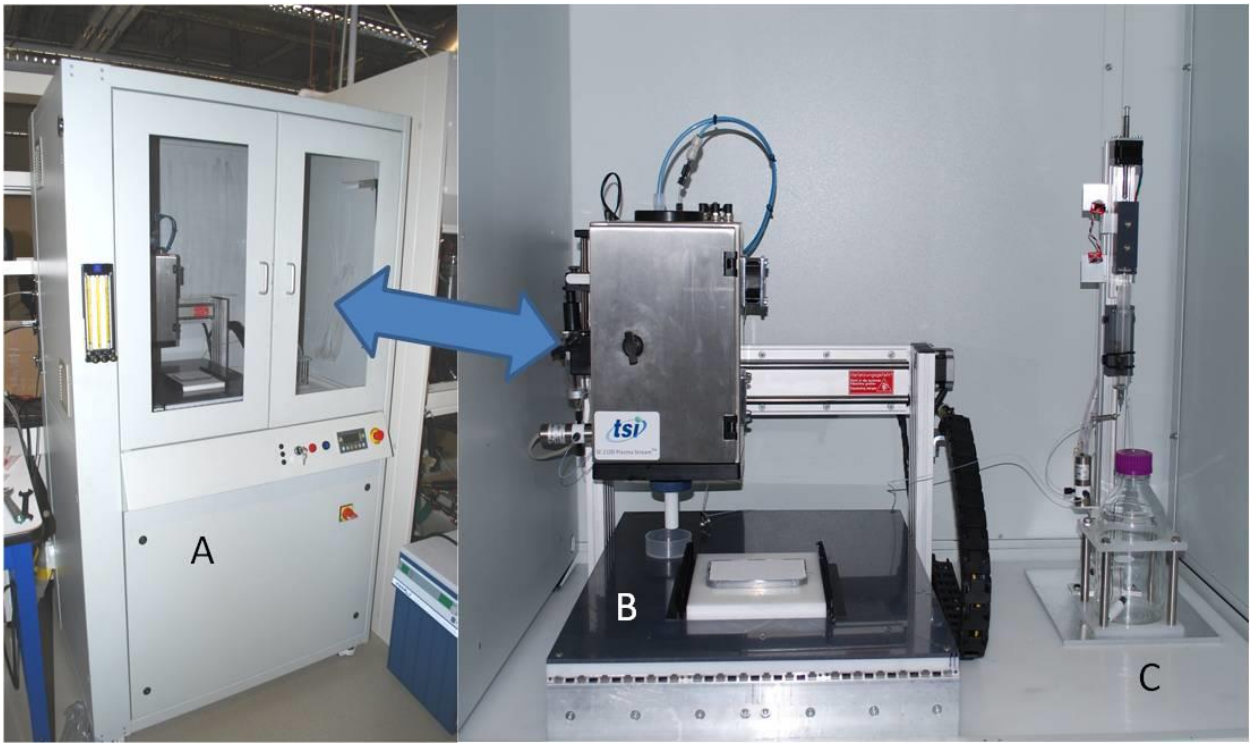


**Figure 3.** A) PlasmaJet installed in the new lab at NDSU, B) Modified deck for fabric substrates, C) Nozzle flushing system

### 2.1.3 Accomplishments and Conclusions

One of the most difficult challenges posed to any antimicrobial materials development program is the ability to efficiently and effectively assess the efficacy of novel concepts and technologies. In most instances, materials scientists are forced to rely on traditional testing methods that, although effective, are oftentimes tedious, labor intensive and time consuming. More importantly, the bulk of these conventional testing approaches are only capable of accommodating relatively small sample volumes (i.e., one or two at a time). In the context of the present program, a testing approach amendable to the evaluation of large numbers of samples in a short period of time was desired, as several antimicrobial approaches based on rather large experimental designs were envisioned at the outset of this project.

To meet this need, a high-throughput screening workflow was successfully developed during the first year of this project. The implemented workflow was designed and constructed to streamline

antimicrobial efficacy assessments through the use of multi-component arrays—including fabrics and rigid materials—that enabled bacterial aerosols to be quickly deposited and rapidly assessed for growth inhibition with minimal, hands-on processing and analysis steps. A series of abrasion and washing protocols were also developed to ascertain the durability and/or long term effectiveness of the antimicrobial treatments when applied to fabrics. In year two of this project, several improvements were made to the screening workflow, including hardware upgrades to the automated aerosolization apparatus to improve the uniformity of bacterial depositions and modifications to the fabric array testing format to improve quantification of bacterial growth. As indicated in section 2.1.2, the PlasmaJet coating platform was successfully installed at NDSU in the final year of this project and has been optimized to apply nano-thin antimicrobial treatments to swatches of fabric. With both the PlasmaJet and antimicrobial screening workflow now firmly in place, NDSU is ideally positioned to continue on in its mission to provide cutting-edge, antimicrobial materials development support and efficacy testing services to both the U.S. Army Research Office and the U.S. Department of Defense.

## 2.2 The Role of Coating Formulation on Coating Performance

### 2.2.1 Acrylic Reactive Monomers Containing Quaternary Ammonium Salts

Acrylate and methacrylate reactive monomers containing quaternary ammonium salt as antimicrobial group were synthesized by solventless reaction between commercially available acrylate and methacrylate monomers with iodoalkane, as described in Figure 4.

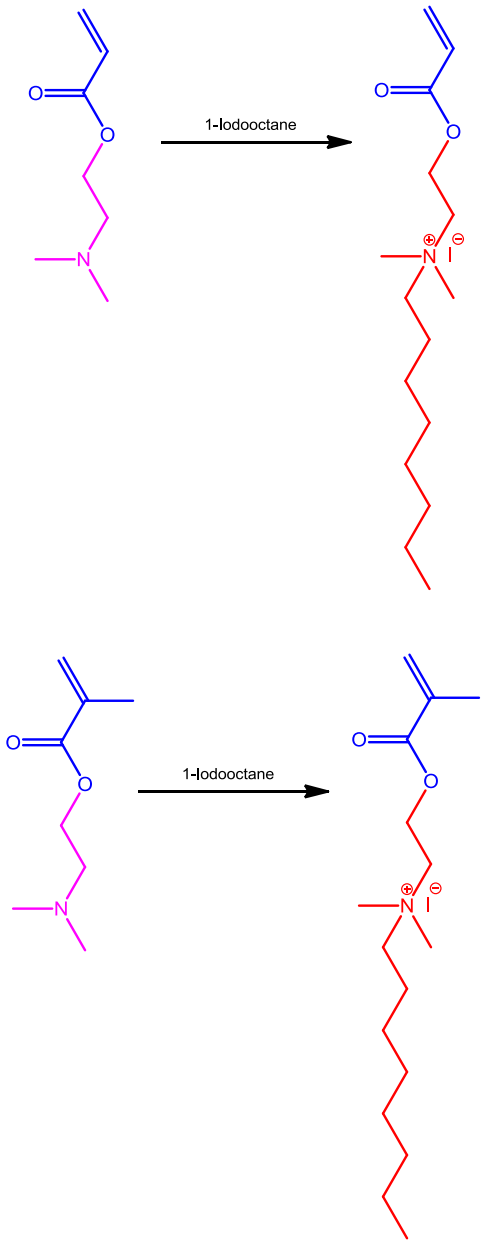


**Figure 4.** Synthesis of antimicrobial acrylate and methacrylate reactive monomers.

Prepared materials were supplied to Triton for further deposition on aluminum and glass substrates using APPLD. Since the reactive groups possessed by the monomers are not likely to chemically bind to these kinds of substrates, thermal post-curing was performed to ensure formation of crosslinked coatings on glass or aluminum surfaces. The growth reduction of *E. coli.*in comparison to untreated substrate is presented in Figure 5.

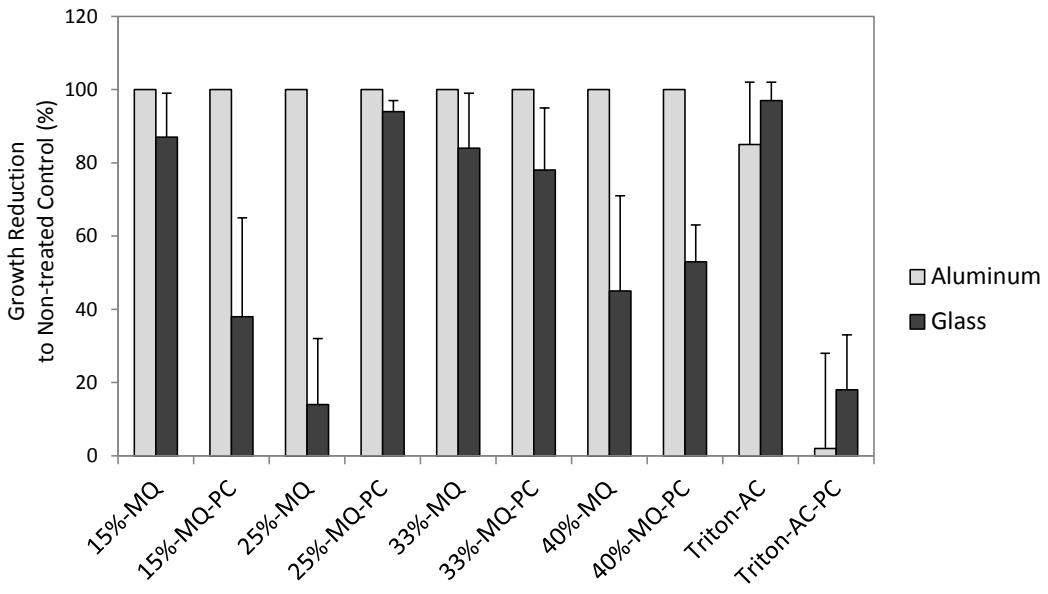


**Figure 5.** *E. coli* growth reduction of substrates treated with quat methacrylate monomer from different concentrations using APPLD.

Antimicrobial assay showed that quat monomer preserves its antimicrobial activity after APPLD process and is very effective on aluminum substrates, while its effectiveness on the glass significantly drops and does not depend on solution concentration or thermal treatment after deposition.

It was of initial interest to utilize polymeric antimicrobial compounds for coatings obtained by APPLD since such approach would produce more durable coatings by maximizing the number ofof reactive groups per chain as well as antimicrobial groups. As it was described in the previous report, an array of allyl-functionalized quatpoly(meth)acrylates was synthesized and polyester textile samples coated with prepared formulations were evaluated for antimicrobial performance. Some of the systematically varied compositions possessed excellent antimicrobial properties and demonstrated very good durability withstanding multiple scrubbing cycles without losing antimicrobial activity. However, those results were obtained by preparing the coatings via UV curing.

In order to establish APPLD conditions for spraying more viscous polymeric compounds, non-reactive and reactive (with allyl groups) model oligomers (designated NRQO and RQO, respectively) both containing 30 mol.% of quaternary ammonium groups were synthesized, as shown in Figure 6. The substrate used for APPLD deposition in this case was a polyester fabric glued on polycarbonate substrate which was later sprayed with bacteria and evaluated for antimicrobial activity. The layout of the deposited coatings is presented in Figure 7. The

prepared oligomers as well as quatacrylate monomer (QM) as a control were deposited using APPLD using a varying number of passes from 1 to 4.

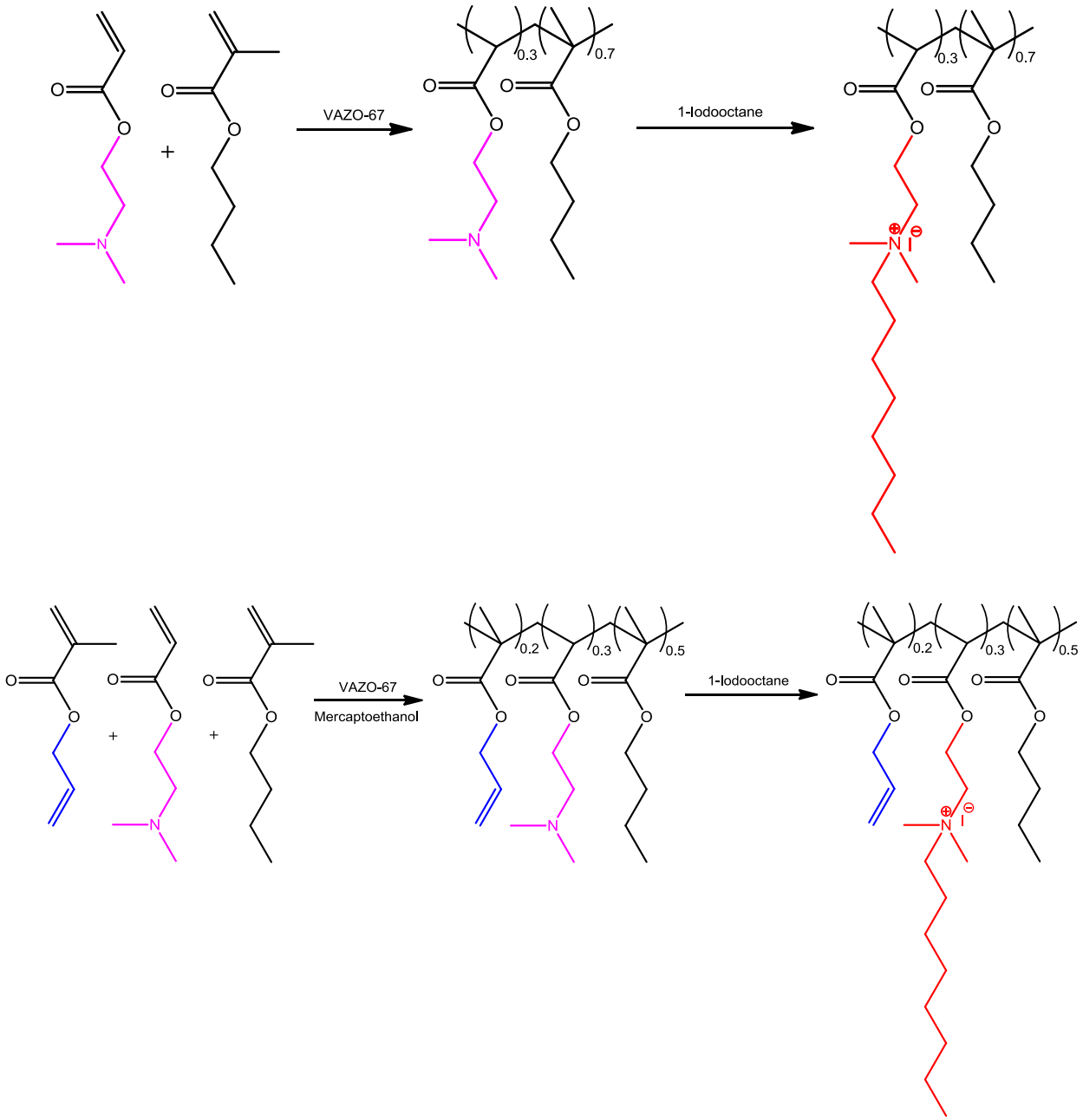


**Figure 6.** Synthetic scheme of NRQO and RQO.

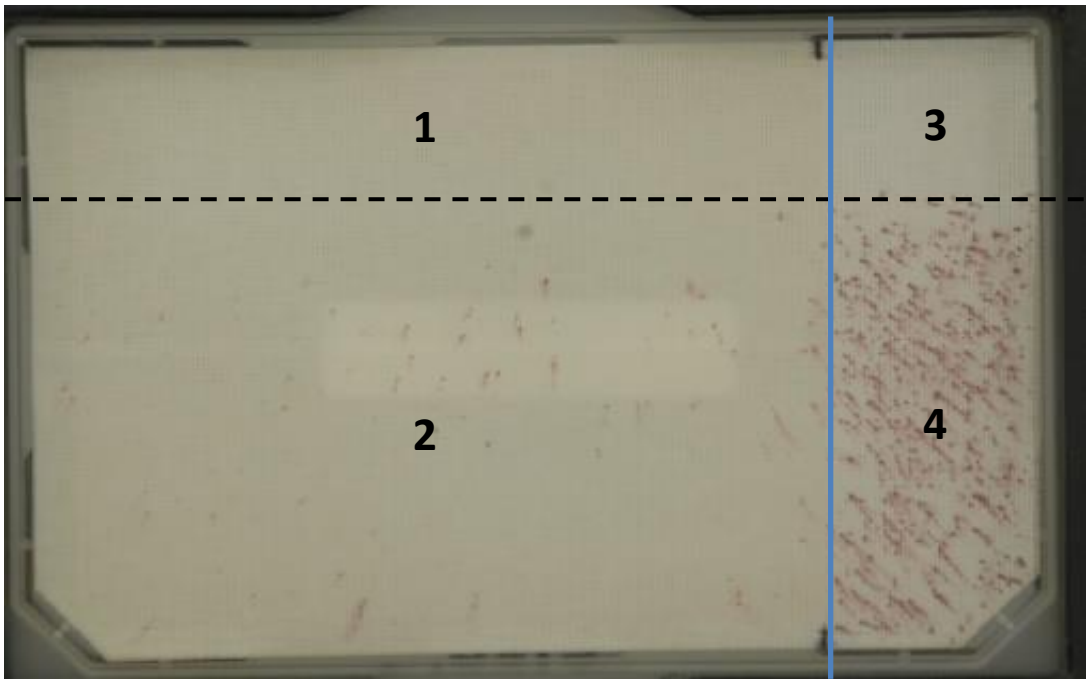


**Figure 7.** Layout of APPLD treated textile samples. Depicted regions: 1 -coated, not sprayed with bacteria, 2 -coated, sprayed with bacteria, 3 -not coated, not sprayed with bacteria, 4 -not coated, sprayed with bacteria.

Figure 8 represents appearance of antimicrobial activity of the fabric samples treated with QM monomer, NRQO, and RQO with the same effective concentration of quat groups. It can be seen that onset of antimicrobial activity is better for QM after one pass, however, after four passes all of the materials demonstrate very good antimicrobial activity towards *E. coli.*

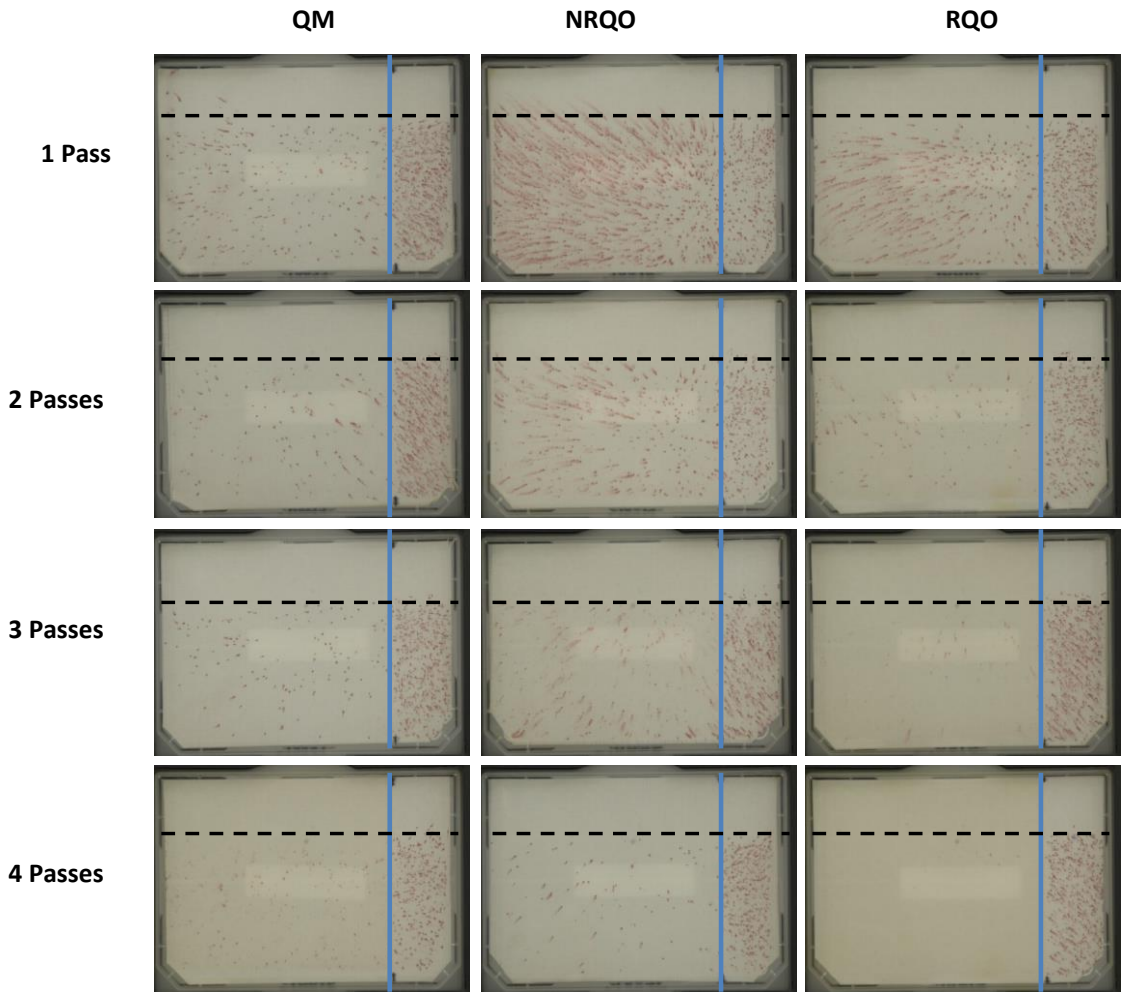


**Figure 8.** Antimicrobial acitivity of QM, NRQO, and RQO deposited on textile samples using varied number of passes using APPLD.

To establish reproducibility of the deposition and to conduct durability tests, these materials were deposited using four passes of APPLD on five replicate textile swatches. However, this time coatings demonstrated much less antimicrobial activity (Figure 9). Initial investigations showed that during nebulization right after the solution with higher molecular weight species was coming out of APPLD nozzle, oligomers tended to deposit on protective Teflon tube while the solvent evaporated, which caused non-uniform deposition and in many cases significant fraction of antimicrobial material did not reach the substrate. Change of solvent to higher boiling point ones did not improve the situation. As APPLD machine has been recently transferred onto NDSU facility, the future plans are to investigate different configurations and parameters of this equipment such as diameter and material of the protective tube, nozzle to substrate distance, nebulizing gas pressure, plasma power, etc. in order to achieve consistently effective deposition of polymeric compounds.

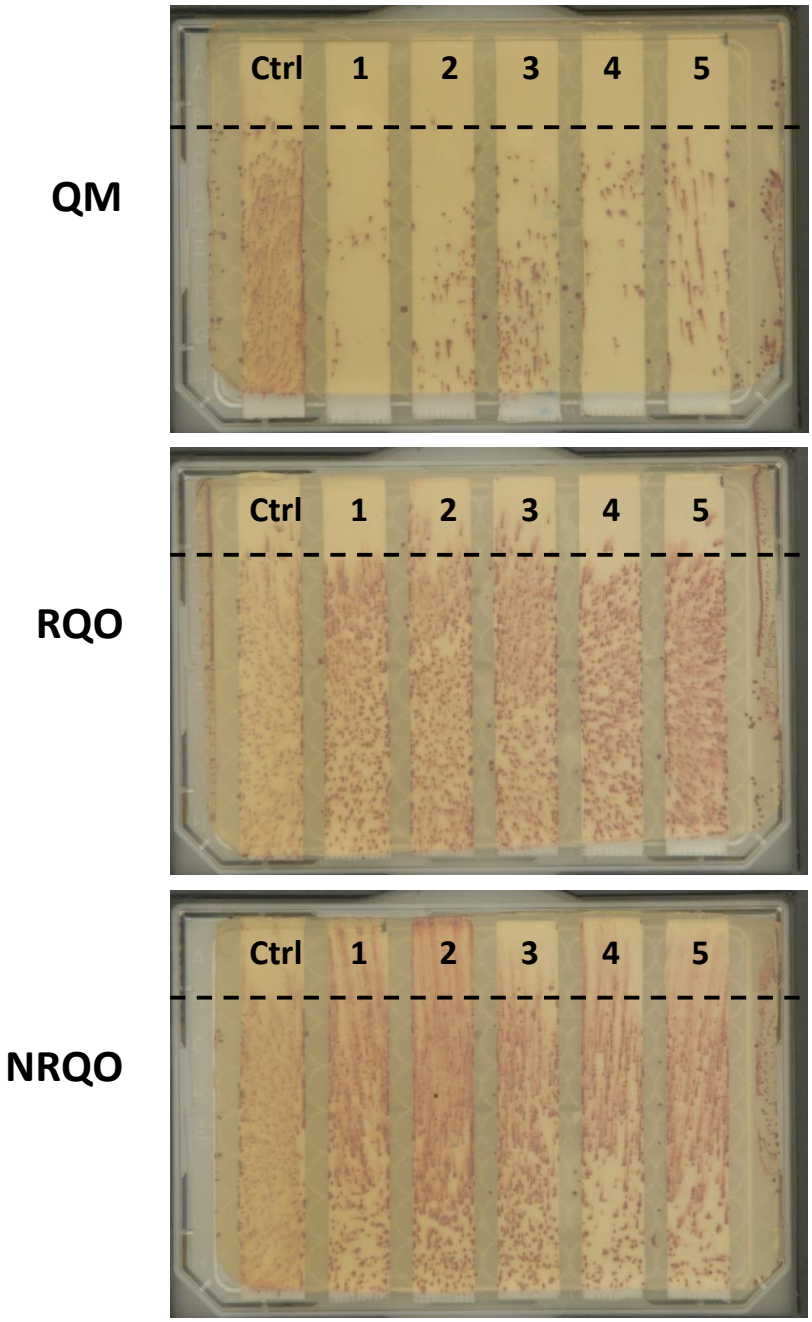


**Figure 9.** Antimicrobial evaluation of five replicate swatches (*E. coli*.).

### 2.2.2 Polyvinyl Oligomers Containing Quaternary Ammonium Salts

As shown in Figure 10, the synthetic process for producing polyvinyl ether oligomers possessing both quaternary ammonium salt (QAS) groups and allyl groups consisted of: (1) the synthesis of 2-iodoethyl vinyl ether from commercially available 2-chloroethyl vinyl ether using sodium iodide; (2) cationic polymerization of 2-iodoethy vinyl ether; (3) substitution of some of the iodo

groups with eugenol; and (4) the formation of QAS groups by substitution of iodo groups with a tertiary amine. At the time that these reactive, functional oligomers were produced, the small lab-scale APPLD system was not readily available for processing, so the antimicrobial activity of oligomers that possessed systematic variations in composition were determined by generating crosslinked coatings via thiol-ene crosslinking. The thiol used for crosslinking was pentaerythritol tetra(3- mercaptopropionate).

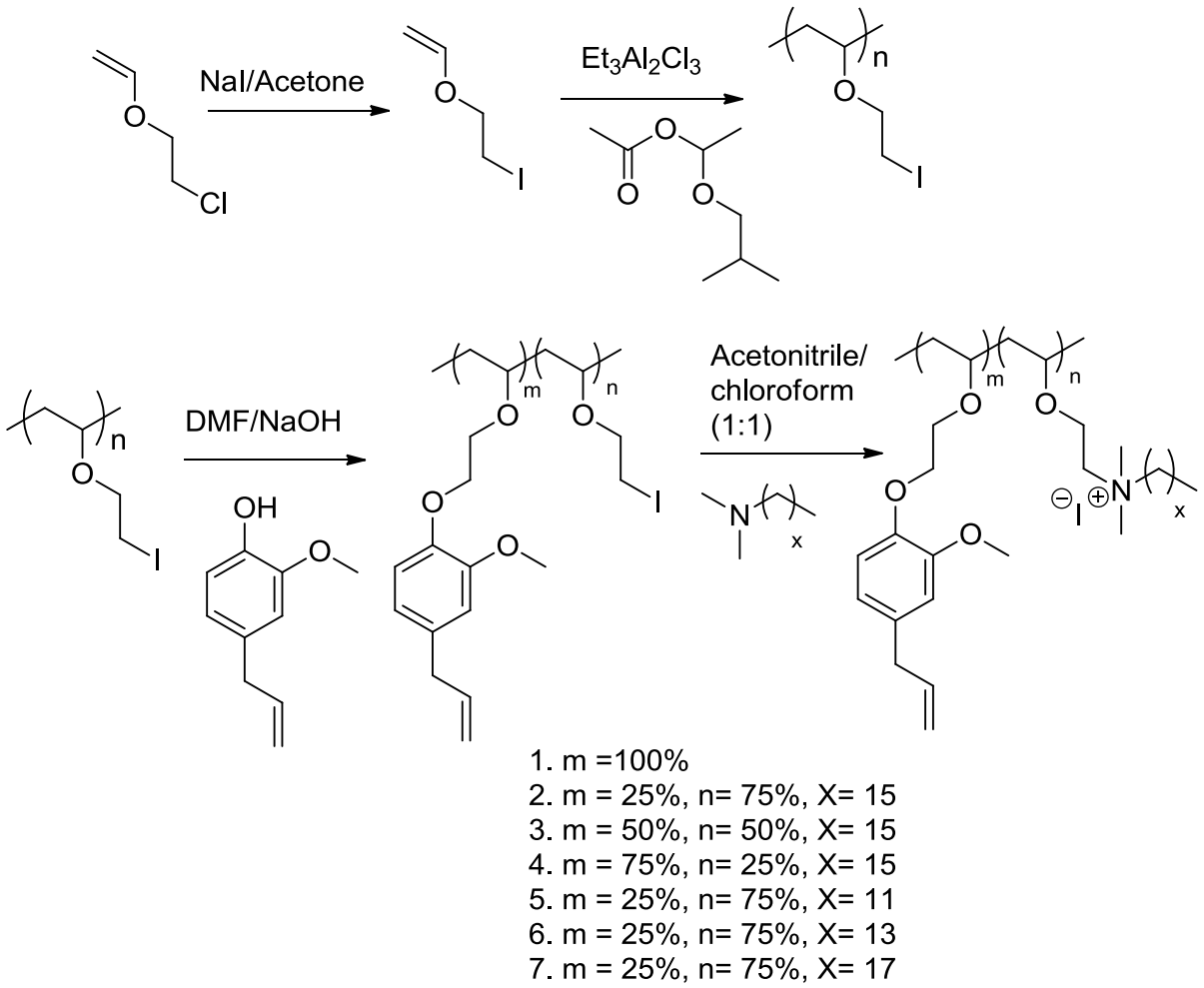


**Figure 10.** The synthetic process used to produce polyvinyl ether oligomers possessing both QAS and allyl groups.

Figures 11 and 12 display images of coatings based on the polyvinyl ether oligomers described in Figure 10 in which the ratio of the eugenol-containing repeat units and the QAS-containing repeat units was varied. The molar ratio of the QAS repeat units to the eugenol (i.e. allyl)-containing repeat units was 0/100, 25/75, 50/50, and 75/25 (poly 1-4). The tertiary amine used for quaternization was dimethylhexadecylamine. The substrate utilized was aluminum discs and crosslinking was achieved by UV-induced thiol-ene coupling reactions. As shown in Figure 11, all of the QAS containing coatings exhibited high antimicrobial activity toward the Gram-

positive bacterium, *Staphylococcus aureus*. For the Gram-negative bacterium, *E. coli*, microbial growth was observed on the coatings containing 25 mole percent QAS groups, while high antimicrobial activity was observed for the coatings containing the higher levels of QAS groups (i.e. 50 mole percent QAS repeat units in the polyvinyl ether oligomer). Thus, similar to the poly(meth)acrylate oligomers, these QAS containing polyvinyl ether oligomers may also be useful for providing antimicrobial activity to fabrics using APPLD.

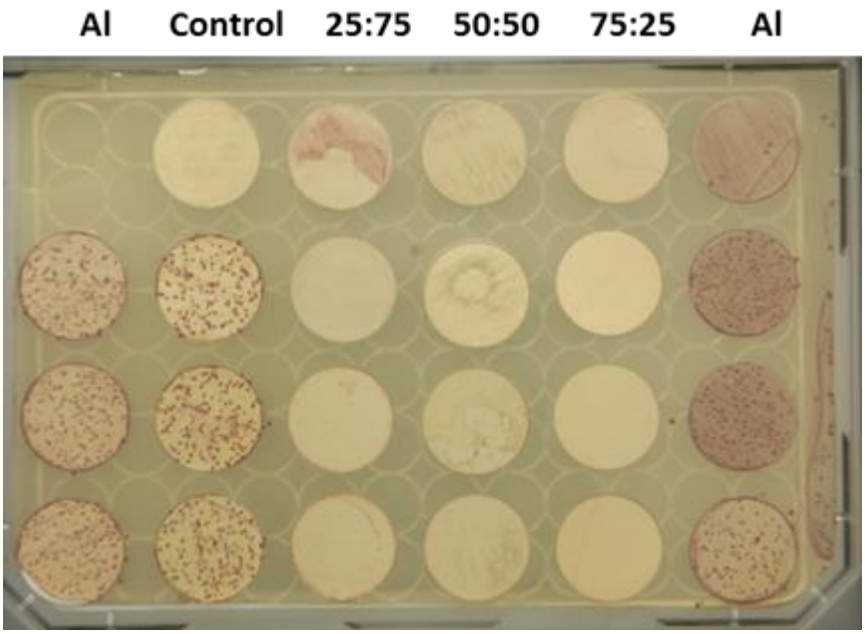


**Figure 11.** An image showing coatings samples based on polyvinyl ether oligomers possessing QAS-functional repeat units and eugenol-containing repeat units. The concentration of QAS-repeat units increases from left-to-right. Antimicrobial activity was determined toward *S. aureus*.

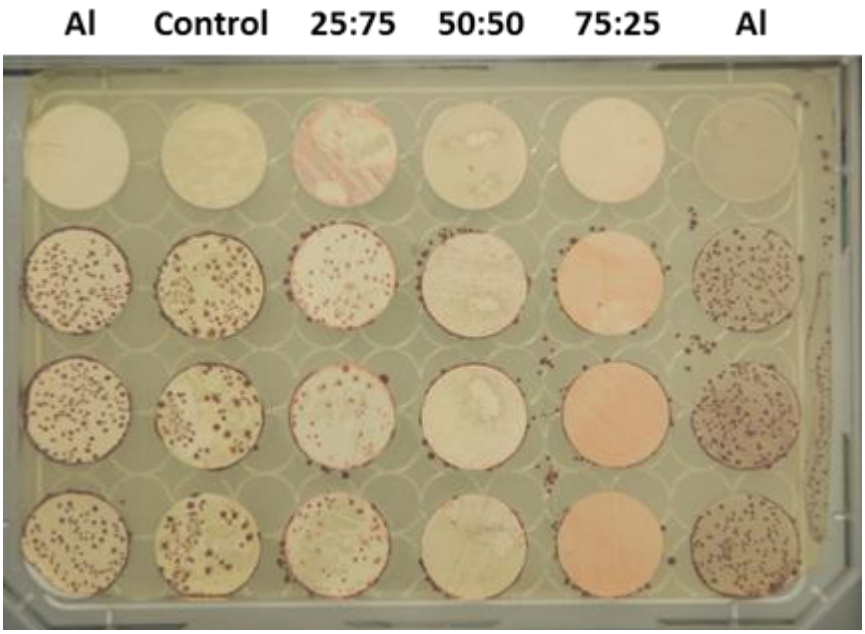


**Figure 12.** An image showing coatings samples based on polyvinyl ether oligomers possessing QAS-functional repeat units and eugenol-containing repeat units. The concentration of QAS-repeat units increases from left-to-right. Antimicrobial activity was determined toward *E. coli*.

As we observed from our initial experiments, coatings based on QAS-functional oligomers are equally effective against gram positive bacteria *S. aureus* with QAS level of 25% to 75% (mole %), but for only higher level (50% and 75%) of QAS-functional oligomers are effective towards gram negative bacteria *E. coli*. However, alkyl chain length of QAS-functional oligomers was not considered for initial experiments. Figure 10, described the synthesis of QAS-functional oligomers with varying alkyl chain length (C12, C13, C16 and C18). The QAS level for all four oligomers were 75 mole percent. The substrate utilized was fabric and crosslinking was achieved by UV-induced thiol-ene coupling reactions. Four variables were employed for current experiments, (1) effect of alkyl chain length (C12, C14, C16 and C18), (2) concentration of solution (0.5 wt%, 1 wt%, 5 wt% and 10 wt%) used for fabrics treatment (3) effect of UV radiation on the coatings (sample with UV and no UV irradiation) (4) effect of washing after UV irradiation (dichloromethane and soap wash). All samples were prepared by soaking the substrate into chloroform solution of QAS-functionalized oligomer and dried with air. Samples were investigated against both gram positive and gram negative bacteria.

Figures 13-16 display images of coatings based on QAS-functional oligomers treated against gram positive bacteria *S. aureus*. Coatings with or without UV (no wash) irradiation exhibited high antimicrobial activity towards gram positive bacteria at all concentrations. The dichloromethane (DCM) or soap washed coatings based on C12 and C14 alkyl chain lengths exhibited antimicrobial activity only with higher concentrations (5% and 10%). On the other

hand, DCM or soap washed coating based on C16 and C18 alkyl chain lengths exhibited antimicrobial activity with a concentration of 1% or above.

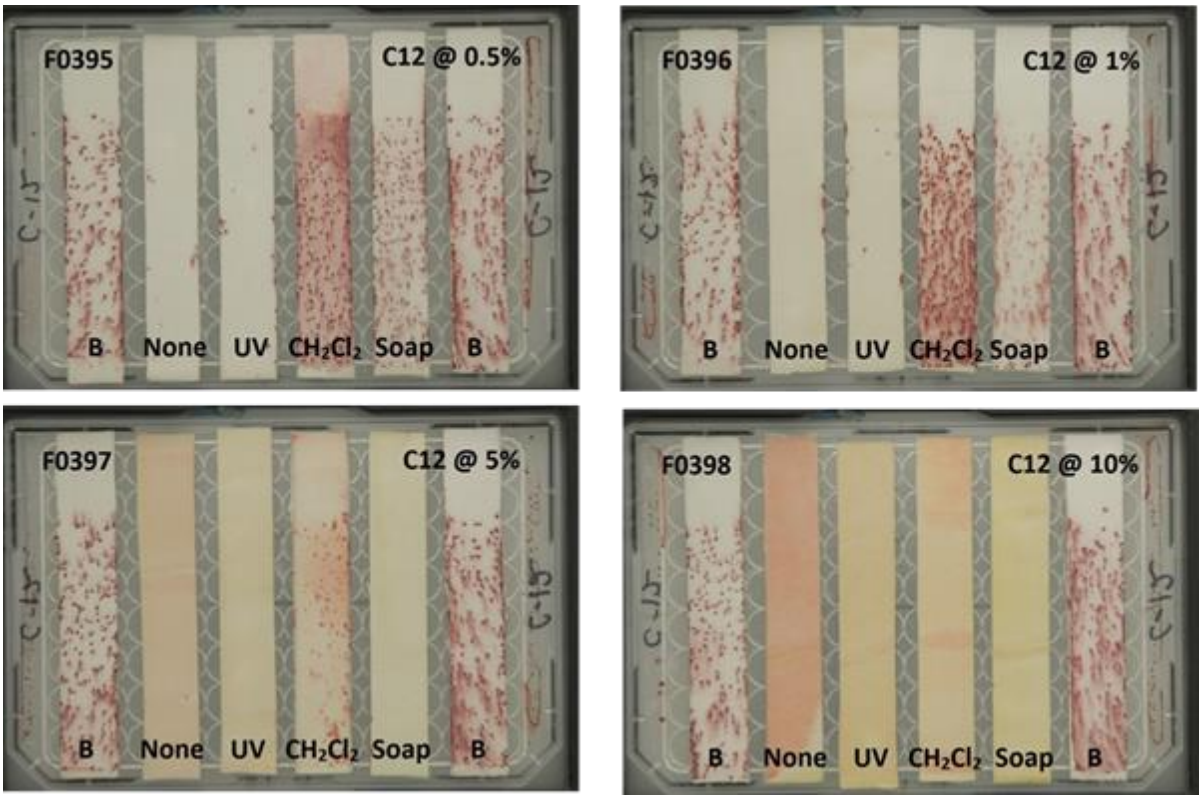


**Figure 13.** An image showing coatings samples based on QAS-functional oligomer (oligomer 5). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *S. aureus*.

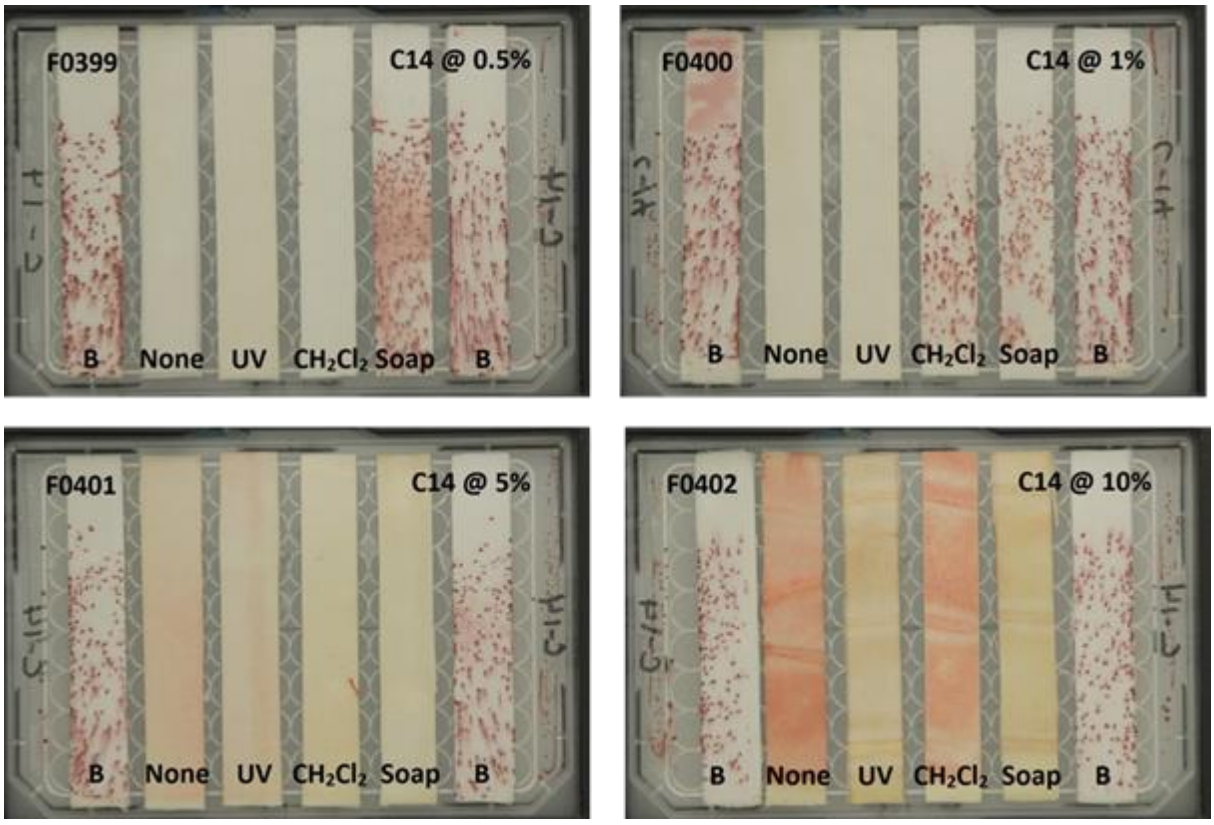


**Figure 14.** An image showing coatings samples based on QAS-functional oligomer (oligomer 6). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *S. aureus*.

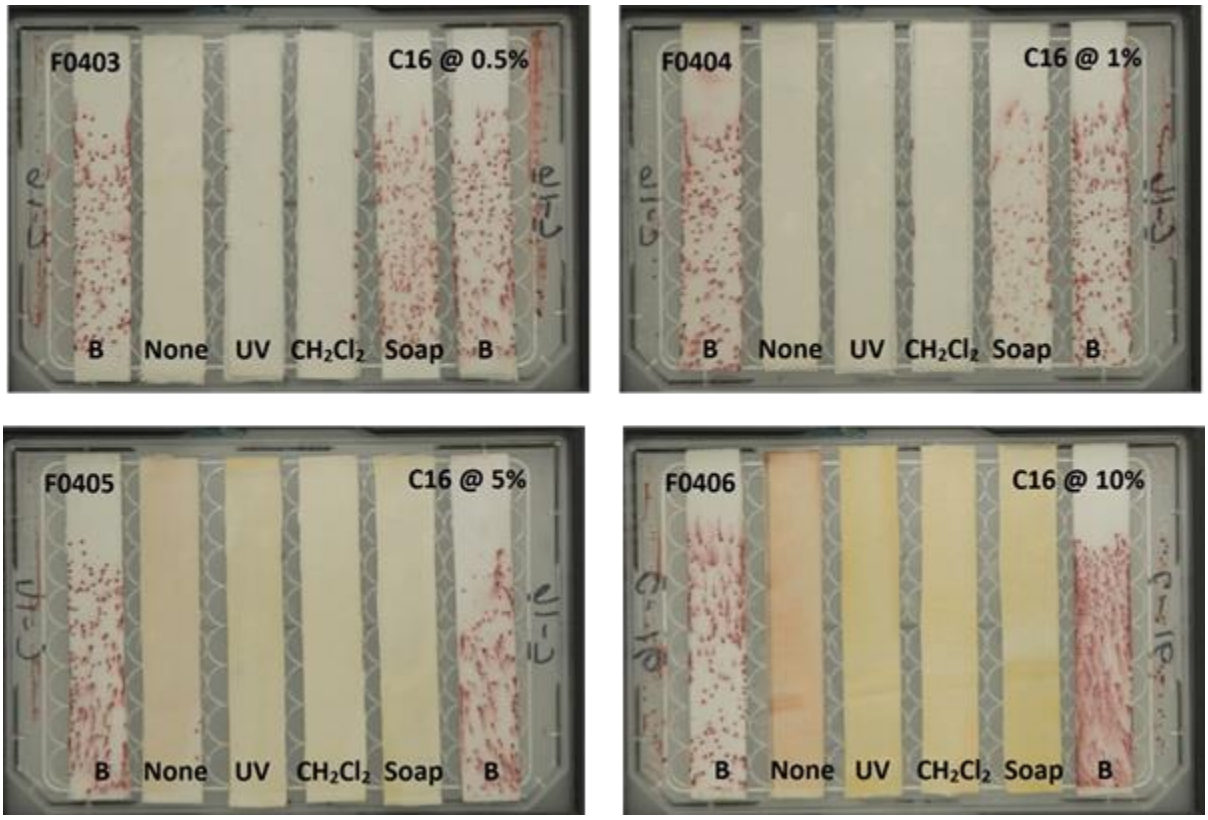


**Figure 15.** An image showing coatings samples based on QAS-functional oligomer (oligomer 2). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *S. aureus*.

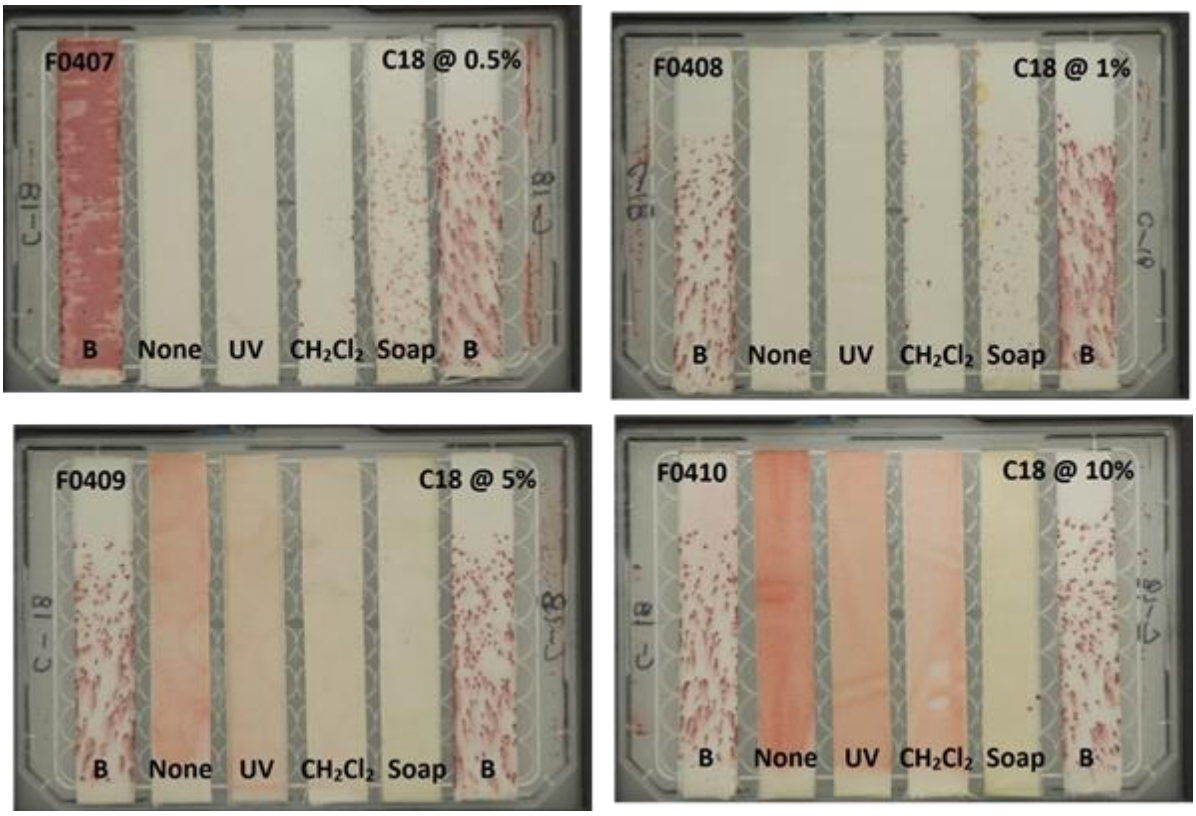


**Figure 16.** An image showing coatings samples based on QAS-functional oligomer (oligomer 7). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *S. aureus*.

Figures 17-20 display images of coatings based on QAS-functional oligomers treated against gram negative bacteria *E. coli*. In general, none of those coatings were effective against gram negative bacteria *E. coli* at low oligomer concentration ( 0.5% or 1%). UV treated and untreated coatings without any washing exhibited antimicrobial activity towards gram negative bacteria with concentration of 5% or above for all oligomer compositions. Although the DCM or soap washed coatings were not effective at concentration level of 5% or below but clearly indicate a major reduction of microorganisms at concentration level of 10%. The coating based on C14 with 10% concentration level was found to be active (100%) towards *E. coli* after washing with DCM or soap.

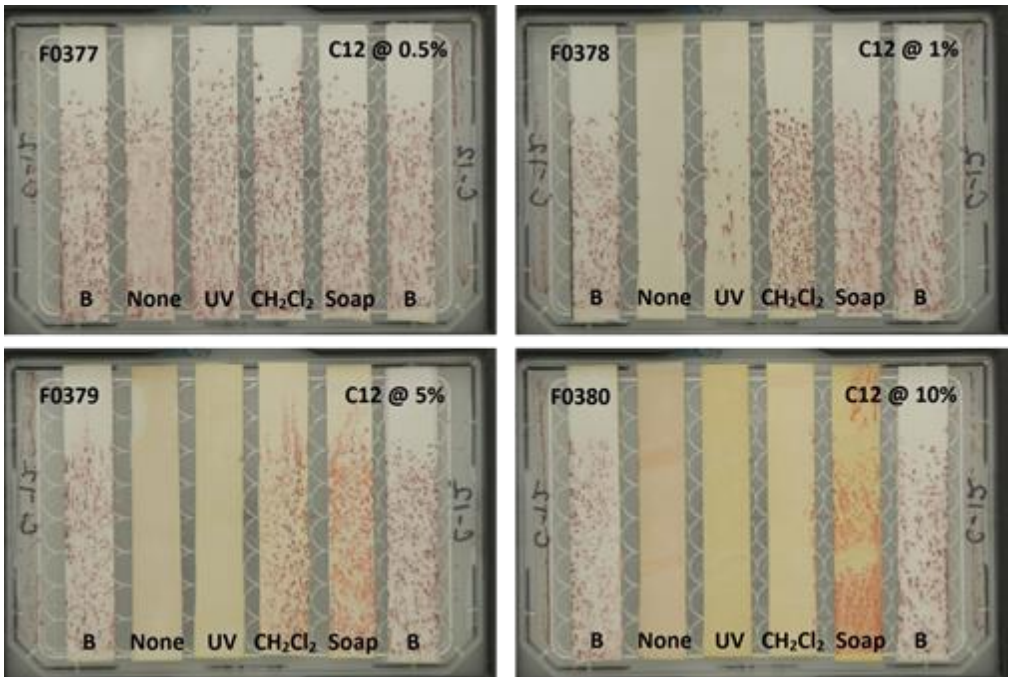


**Figure 17.** An image showing coatings samples based on QAS-functional oligomer (oligomer 5). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *E. coli*.

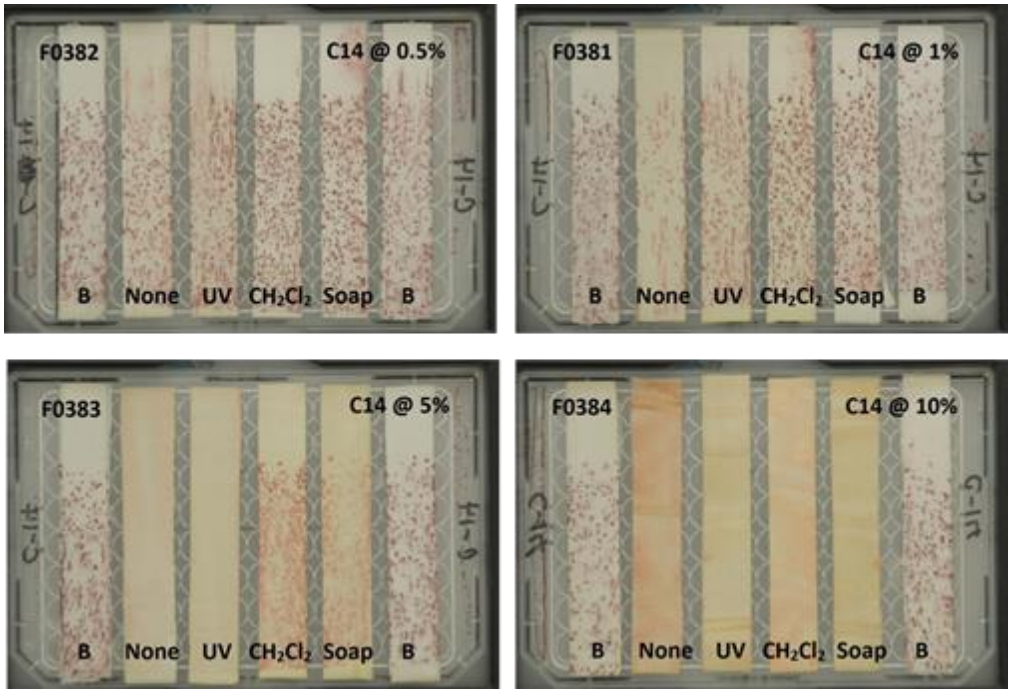

**Figure 18.** An image showing coatings samples based on QAS-functional oligomer (oligomer 6). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *E. coli*.

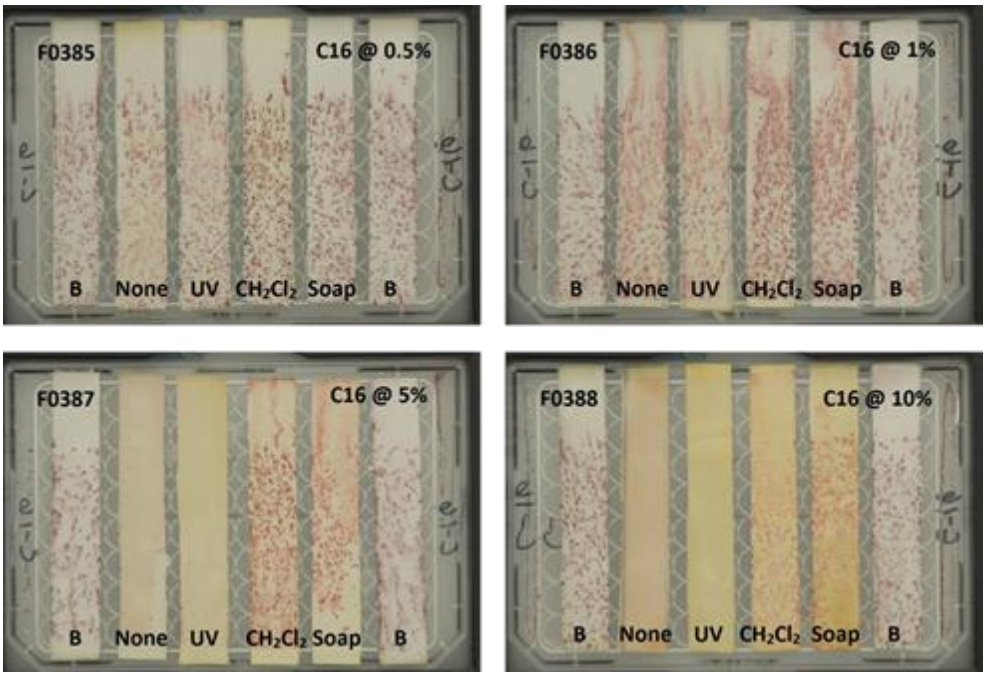


**Figure 19.** An image showing coatings samples based on QAS-functional oligomer (oligomer 2). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *E. coli*.

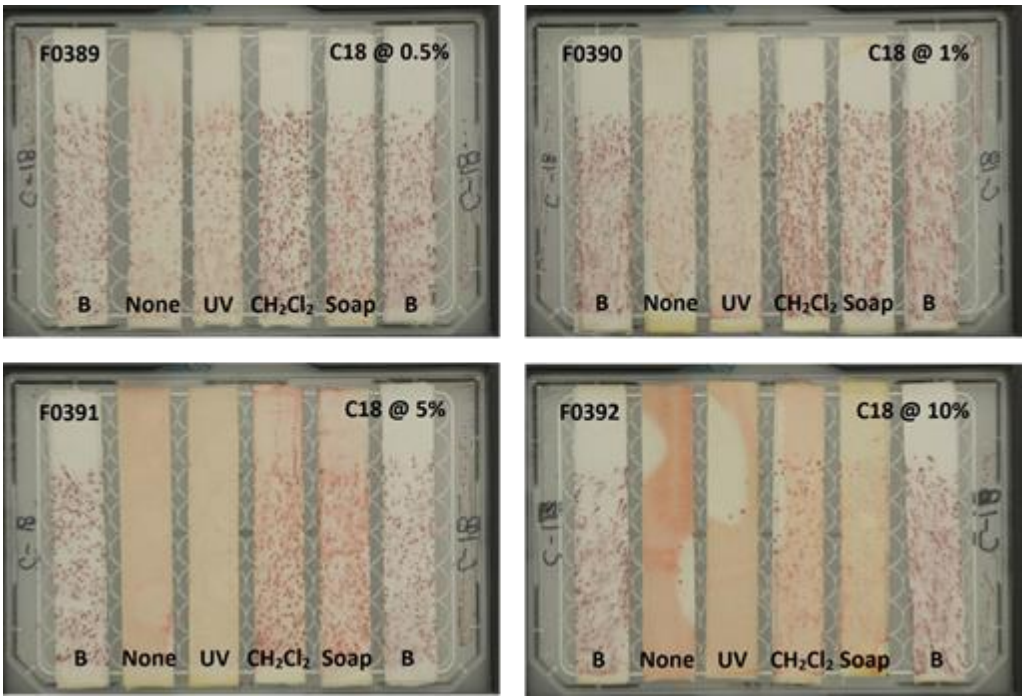


**Figure 20.** An image showing coatings samples based on QAS-functional oligomer (oligomer 2). The concentrations of oligomer in chloroform solvent vary from 0.5% to 10%. Antimicrobial activity was determined toward *E. coli*.

Copolymer derived from soybean oil based monomer and QAS was investigated as antimicrobial coatings. Figure 21 shows the synthesis of copolymer with 50/50 mole ration of soybean oil repeat unit and QAS repeat unit. The tertiary amine used for quaternization was dimethylhexadecylamine. The crosslinking was achieve by the UV induce thiol-ene reaction between QAS-functionalized polymer and pentaerythritol tetra(3- mercaptopropionate). The substrate utilized was aluminium discs and antimicrobial activity was tested towards gram negative bacteria *E. coli* for preliminary experiment. As shown in Figure 22, copolymer with 50/50 mole ratio of soybean oil repeat unit and QAS repeat unit exhibited high antimicrobial activity toward gram negative bacteria. So, it is expected that the coating would be highly effective towards gram positive bacteria.

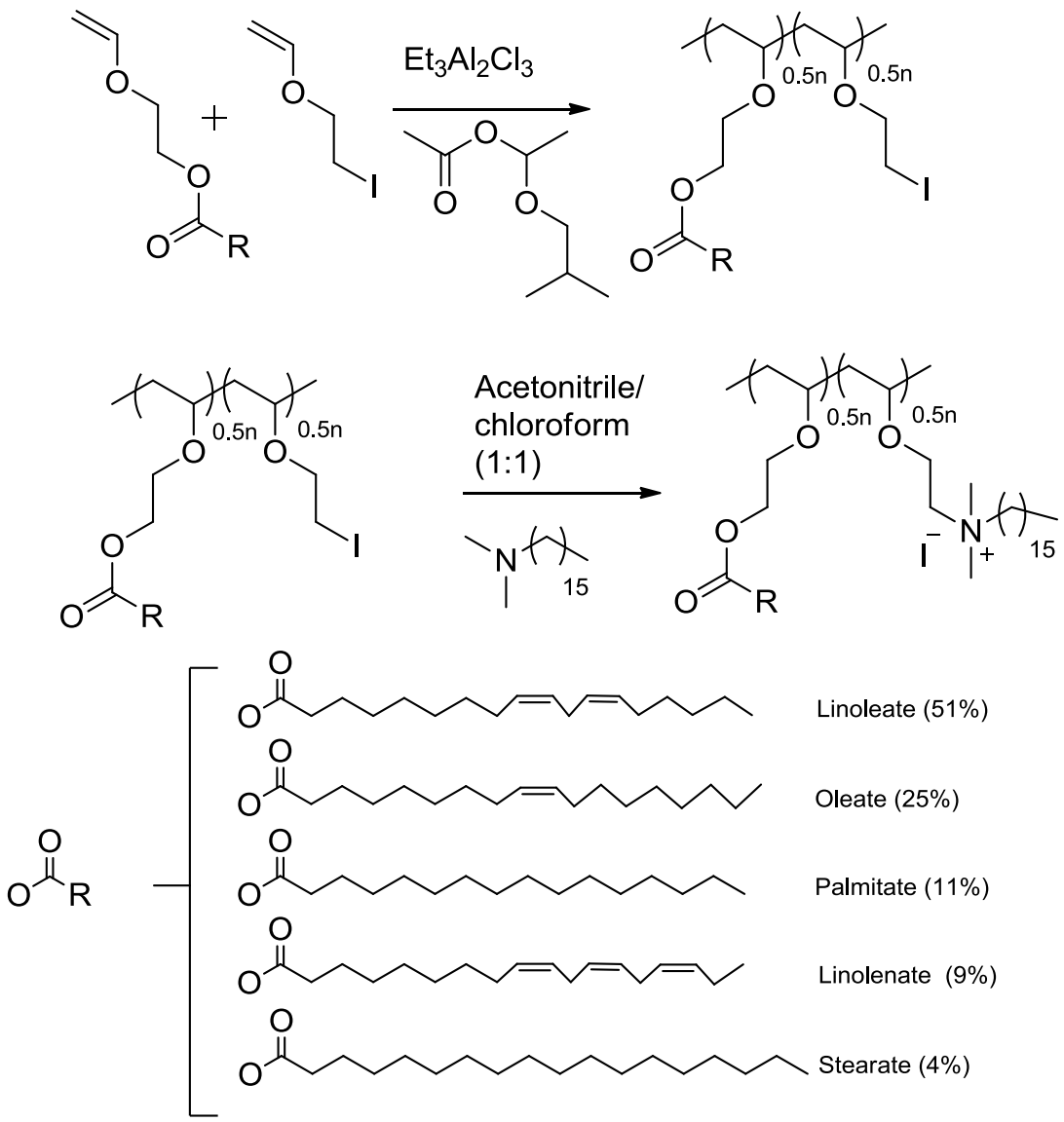


**Figure 21.** The synthetic process used to produce polyvinyl ether oligomers based on soybean monomer possessing both QAS and allyl groups.

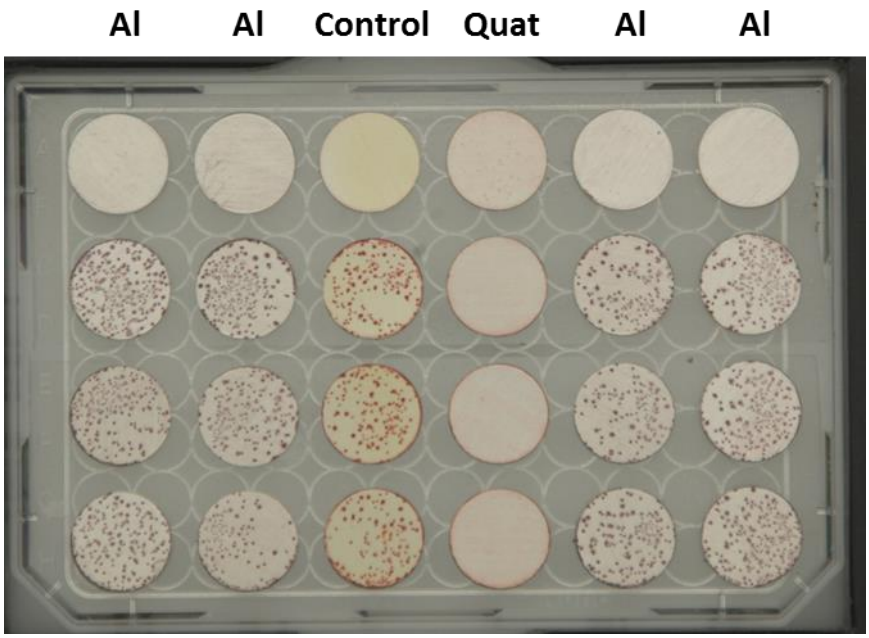


**Figure 22.** An image showing coatings samples based on polyvinyl ether oligomers possessing QAS-functional repeat units and soyate-containing repeat units. Antimicrobial activity was determined toward *E. coli*.

### 2.2.3 Accomplishments and Conclusions

Two material approaches were investigated for the synthesis of reactive precursors for imparting broad-spectrum antimicrobial activity to fabrics. Both approaches involved the use of quaternary ammonium salt (QAS) functional groups for obtaining antimicrobial activity. QAS groups are widely utilized for producing antimicrobial surfaces since they impart antimicrobial activity through a "contact" mechanism via disruption of the bacterial cell wall. One approach consisted of conversion of a tertiary amino-functional acrylate to a QAS-functional acrylate and deposition of the QAS-functional acrylate onto fabric using atmospheric pressure plasma depositon (APPLD). This approach was found to be effective for creating antimicrobial surfaces. The second approach consisted of the synthesis of both polyacrylate and polyvinyl ether oligomers that possessed both pendent QAS groups and pendent allyl groups. It was hypothesized that the use of these oligomers instead of a monomeric species would enable better grafting of the QAS groups to the substrate using APPLD because of the higher number of free radically-reactive functional groups per molecule associated with the oligomers. Unfortunately, the higher viscosity of these oligomeric materials made processing using APPLD difficult. Nonetheless, treating fabric using these oligomers in conjunction with a dipping or spraying process and subsequently crosslinking the oligomers using UV light resulted in antimicrobial surfaces. A number of compositional factors were investigated including the concentration of the QAS

functional groups, the length of the alkyl chain associated with the QAS groups, the concentration of the oligomer solution, and the extent of the UV exposure used for crosslinking.

## 3.0 Summary of Technical Progress – Triton Systems, Inc.

Triton Systems Inc. tasks aimed at developing antimicrobial functional coatings deposited via an atmospheric pressure plasma liquid deposition process called Invexus™. Invexus™ is a novel plasma aided surface functionalization technique for deposition of nanometer thick functional coatings/films onto various substrates under atmospheric temperature and pressure. The novelty of this process is that the coatings are deposited using liquid precursor formulations which are activated / polymerized / deposited under a cold inert gas plasma. The ability to utilize liquid precursors gives the flexibility to choose from a wide variety of precursor molecules and hence tailor the surface properties as needed. Further, since the deposition is under near ambient conditions, the fragmentation of the precursor molecules within the plasma is generally minimal, resulting in transfer of the properties of the precursors to the substrate surface. Deposition of the coatings simultaneously in the presence of the plasma is also expected to chemically bind the coatings to the substrate, thereby resulting in coatings with improved durability.

In this project, Triton accomplished the following milestones:

1) Established a plasma coating capability at Triton-ND facility for both flexible webs and rigid 3D substrates
2) Built and transferred coating equipment and know-how to NDSU for coating development using High Throughput Screening workflow
3) Formulated antimicrobial coating precursors and produced antimicrobial treated fabric substrates of interest to the military

Triton built and installed two fully functional atmospheric plasma coating lines designed specifically for treating flexible substrates such as textiles and films as well as rigid 3D components. A brief description of the two treatment platforms is given below:

### 3.1 Roll-to-Roll Textile Treatment Line (RC1000™)

Triton designed, built, and assembled an industrial scale roll-to-roll atmospheric pressure coating line, RC1000™ located at our application development center in Fargo, ND. RC1000™ is a stand-alone (pictured in Figure 23), roll-to-roll system for flexible web materials up to 72 inches wide, ideally suited for full scale product development, initial production orders, or mainstream production. It has state-of-the-art automation, control, and diagnostics with versatile web handling for a wide variety of textile substrates (woven, non-woven, knit) and flexible plastic films.

**Figure 23.** Picture of Triton's Industrial Scale Roll-to-Roll Atmospheric Pressure Plasma Coating Line (RC1000™) for webs up to 72" wide.

## 3.2 Plasmastream Treatment Platform

Plasmastream is a robotic equipment for coating individual components or devices and other 3D rigid substrates. It can be simply described as a computer controlled x-y-z table and a plasma deposition head where the plasma generation and precursor atomization takes place. The activated liquid aerosol is directed and deposited onto the substrate by the plasma jet generated in the plasma head. It is shown in Figure 24.

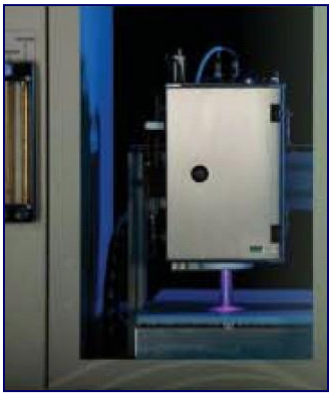

**Figure 14**. Plasmastream coating platform for coating rigid substrates

## 3.3 Building and Transfer of Coating Equipment to NDSU

Triton completed building of a Plasmastream coating platform for use with NDSU's High Throughput Screening workflow. A Plasmastream equipment built earlier by Dow Corning, but was not functioning was rebuilt for this Task. This involved installation of the CnC drive, building and integration of the plasma head, installation of gas distribution system, and control systems for the machine. The machine was extensively tested for its robustness and operationality. The machine was transferred to NDSU in April 2013 for coating work in combination with their high throughput screening process for development of antimicrobial coatings. Subsequently, Mr. John Lovassen, Staff Engineer at Triton Systems Inc trained NDSU staff in the operation of the equipment. A picture of the finished machine is shown in Figure 25.

**Figure 25.** Plasmastream equipment for coating rigid substrates transferred to NDSU.

## 3.4 Anti-microbial coatings for textiles using Invexus™ atmospheric pressure plasma deposition

Triton Systems Inc tasks aimed at developing antimicrobial functional coatings deposited via its Invexus™ atmospheric pressure plasma liquid deposition process. The development work involved formulation development, equipment modification, process optimization, and testing/property validation.

Triton formulated antimicrobial coating solutions using biguanide as the active ingredient using suitable binders and crosslinking monomers and deposited the same onto nylon and polyester textiles using our 72" wide industrial scale roll-to-roll atmospheric pressure plasma coater, RC1000™. A schematic of the coating line is shown in Figure 26. The line consists of 3 main zones: a pre-treatment plasma zone into which the fabric enters first, followed by a coating zone where the liquid coating precursor containing the active biocide is sprayed onto the substrate, and a final curing plasma zone where the sprayed coating is cured/bonded on to the substrate. The experiments in this task were used to determine optimum process conditions for obtaining uniformly deposited active antimicrobial coatings across the entire width of the web as well as refine the design and robustness of the roll-to-roll coating system.

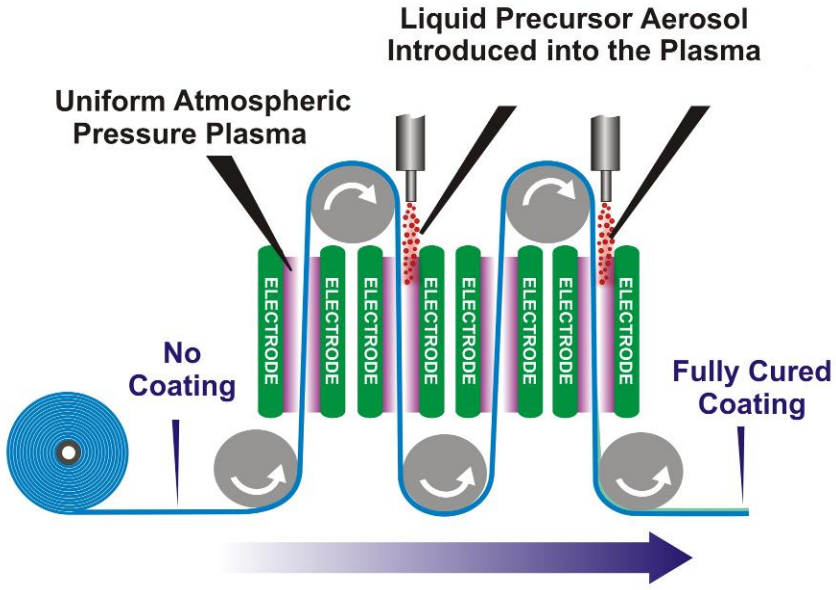


**Figure 26.** Schematic of the plasma coating process.

Through several iterative experiments, the effect of precursor flow rate, line speed, coating level, and aerosolizing mechanism set up on the coating deposition were optimized. The experimental work enabled optimization of the antimicrobial coatings for textiles. Final samples TSI# KJ-44-1 and KJ-45-1, deposited on nylon and polyester based fabric, respectively, were sent to North Dakota State University and analyzed for their bioefficacy using the High Throughput Biological Screening Workflow tool against *Escherichia coli*. Both samples of KJ1-44-1 and KJ1-45-1 were found to be very bioactive and showed almost complete inhibition of *E. coli* growth. Further, the coated fabrics were found to have good bioefficacy across the entire width confirming a fairly uniform coating deposition on both substrates.

## 3.5 Anti-microbial coatings for Rigid Substrates using Invexus™ atmospheric pressure plasma deposition

In addition to the textile treatments, Triton also formulated coating solutions using quat polymers synthesized by NDSU and deposited the same onto rigid substrates (glass and aluminum discs) using our Invexus™ coating technology. All coatings were performed using the Plasmastream workstation, and glass and aluminum were used as the candidate substrates of interest. The experiments focused on studying the effect of process parameters such as plasma power, gas flow rates, and precursor flow rates on the antimicrobial activity of the coated substrates. All coated specimens were sent to NDSU for efficacy testing.

## 3.6 Accomplishments and Conclusions

Especially for textiles, typical treatment is performed via wet chemical pad-dry techniques that involves dipping the fabric in a bath of coating solution, removing excess liquid, and subsequent drying/curing in a series of high temperature ovens. The process is energy intensive, utilizes solvents, and generates liquid waste. Triton's Invexus™ process, on the other hand, transfers the desired functional groups from a liquid precursor, as a well-adhered, nanothin treatment onto the substrate surface through a one-step "green" atmospheric plasma process. The process does not use solvents, uses minimal amounts of liquid precursors, and does not require high temperature ovens to cure the deposited coatings.

Feasibility of Triton's novel Invexus™ plasma treatment process for treatment of various substrates with antimicrobial coatings was successfully demonstrated. Two candidate fabrics of importance to the US Military, namely Nylon which is a typical fiber used in several military clothing and polyester fabrics that are widely used in making liners in soft wall shelters used in Deployable Medical Systems (DEPMEDS), were successfully treated with the formulated antimicrobial coatings at full width industrial scale. These coatings were found to successfully inhibit the growth of *E. coli* bacteria when tested using the high throughput screening workflow. Additionally, preliminary work on development of quaternary ammonium based antimicrobial coatings for rigid substrates was demonstrated through coating work on glass and aluminum substrates. Results from testing of the coated samples showed the quat based polymeric coatings were successful in inhibiting the activity if *Staphylococcus aureus*. In summary, the work in this program demonstrated the feasibility of Triton's Invexus™ treatment process to be able to deposit efficacious antimicrobial coatings on various substrates with relevance to the individual and collective protection equipment.

# 4.0 Program Management

Dr. Bret Chisholm, Associate Director for Combinatorial Chemistry at NDSU Center for Nanoscale Science and Engineering (CNSE) serves as the principal investigator for this project. Mr. Shane Stafslien, NDSU CNSE, leads efforts associated with the high-throughput characterization of antimicrobial properties, while Mr. James Bahr, NDSU CNSE, leads efforts associated with the integration of APPLD into the combinatorial/high-throughput workflow at NDSU. Dr. Arjan Giaya, VP of Technology at Triton Systems, who is responsible for the development of new materials and technologies for chem/bio applications and their transition to commercial phases, coordinates activities at Triton. Other key contributors from Triton include Dr. Yoojeong Kim, an experienced engineer in the area of antimicrobials, and Mr. Apoorva Shah, a process development engineer.